Program/Abstract # 346
Formation and interpretation of the BMP morphogen gradient in the Drosophila embryo
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The early Drosophila embryo uses a step-gradient of Bone Morphogenetic Protein (BMP) to define spatial coordinates along the dorsal–ventral axis. Formation of the step-gradient requires a redistribution of BMP from the lateral domains to the dorsal midline (DM). This occurs post-translationally via a conserved shuttling mechanism that involves the activity of the BMP-binding protein Short Gastrulation (Sog), and the protease Tolloid (Tld). The vertebrate counterpart of Sog, Chordin (Chd), cannot promote long-range shuttling of BMP when introduced into flies. Molecularly, these two proteins differ in that Sog processing by Tld is dependent on BMP-binding, while Chd processing is not. Here we show that the BMP-dependence for Tld-mediated cleavage, allows Sog to be more effective than Chd at facilitating the long-range transport of BMP. We characterized the Sog processing sites and found that several residues were responsible for making Sog destruction dependent on BMP binding. Replacement of endogenous Sog with "Chd-like" variants changed the steep BMP gradient to a shallower and more variable profile. Such changes in the gradient profile had a major impact on patterning, as it affected cell-fate allocation and tissue size and resulted in increased variability of patterning across individual populations. Further, embryos with the "Chd-like" Sog were less able to compensate for developmental challenges, such as a decreased rearing temperature or a reduced level of BMP. We conclude, therefore, that BMP-dependent Sog processing is fundamental to the long-range facilitated diffusion of the BMP ligand, and thus, formation of the robust bi-stable BMP morphogen gradient, and reliable developmental patterning of the embryo.

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Program/Abstract # 347
Gene regulatory networks in embryos depend on pre-existing spatial coordinates
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The development of metazoan embryos requires the precise spatial deployment of specific cellular functions. This deployment depends on gene regulatory networks (GRNs), which operate downstream of initial spatial inputs (E. H. Davidson, Nature 468 [2010]: 911). Those initial inputs depend, in turn, on pre-existing spatial coordinate systems. In Drosophila oocytes, for example, spatial localization of the earliest-acting elements of the maternal GRN depends on the prior establishment of an anteroposterior body axis by antecedent asymmetries in the ovary. Those asymmetries appear to be derived from cytoskeletal and membrane patterns rather than on DNA sequences, and there is evidence that some cytoskeletal and membrane patterns can be inherited independently of the DNA. I review that evidence, suggest that such patterns provide developmental information that must precede the operation of GRNs, and discuss possible implications of that information for evolutionary theory.

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Program/Abstract # 348
Dpp/BMP pathway regulates maternal mRNA levels to pattern the dorsa–ventral axis in *Drosophila melanogaster* embryo
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Dorsoventral patterning in *Drosophila melanogaster* is regulated by the Toll pathway by modulating Cactus/IkB protein degradation, and thus nuclear Dorsal/NFkB protein levels in the early embryo. In addition to the Toll pathway, through previous genetic and biochemical studies we have shown that the maternal Dpp/BMP pathway regulates nuclear Dorsal levels by controlling Toll-independent degradation of Cactus. The maternal Dpp pathway requires Calpain A to target Cactus degradation and thus to regulate Dorsal levels. Using a Real-time qPCR approach we have shown that the maternal Dpp pathway regulates mRNA levels of Calpain A and Casein Kinase II in the early embryo. These results suggest that maternal Dpp functions through a novel mechanism, since the effects of Dpp blockage are observed before the onset of zygotic transcription. This raises the possibility that the maternal Dpp/BMP pathway acts through post-transcriptional mechanism to regulate stability or degradation of mRNAs. Accordingly, null germline clones for the tkv type I receptor, mad or medea SMADs generate similar defects on the nuclear dorsal gradient and the dorsoventral patterning. In order to define the functional role of maternal Dpp on mRNA levels we have undertaken a microarray based approach. Recent data reveals that we are able to detect variations in mRNA levels in the pre-blastoderm embryo upon blockage of maternal Dpp signals. The results obtained up to now suggest that Dpp regulates maternal mRNA levels in the embryo. We are currently exploring whether some of these maternal mRNAs participate with Dpp to regulate discrete nuclear Dorsal levels and thus DV patterning. Grant sponsors: CNPq, Pronex, FAPERJ and INCT-INEM.

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Program/Abstract # 349
Characterization of the feedback circuit driving robust BMP signaling during embryonic dorsal–ventral patterning in Drosophila
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Bone Morphogenetic Protein (BMP) signaling is essential for patterning the dorsal–ventral (D/V) axis in the early Drosophila embryo. The BMP ligand Dpp is secreted over the dorsal 40% of the embryo prior to gastrulation but signals only in the dorsal-most 10% of cells at the onset of gastrulation. Two processes cooperate to produce an intense, sharply defined BMP signaling domain. First, Dpp is concentrated in the dorsal domain by directed, long-range extracellular transport. Second, an intracellular positive feedback circuit promotes future ligand binding as a function of previous signaling strength. We have now identified components of the positive feedback circuit. The BMP target gene eiger, which encodes a TNF-α homologue that activates the Jun N-terminal kinase cascade, is required to achieve wild-type levels of BMP signaling. Conversely, crossveinless-2 (cv-2), which encodes a non-signaling BMP binding protein that is expressed in the same domain as dpp, acts to sequester BMP ligands during D/V patterning. Loss of both eiger and cv-2 renders D/V patterning very non-robust, with extreme variability in the intensity and spatial extent of BMP signaling. Preliminary investigations into other Drosophila species, found in relatively invariant environments, have shown that these species lack components of the feedback circuitry. We propose that positive feedback